

Determination of Water-borne Selenium Assimilation Efficiency

Brine Shrimp Kinetics Study

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DATE: September 26, 2007

1. Follow SOP for determination of water-borne selenium uptake steps 1-9. Use adult fully grown individuals.
2. Allow for a total of 48 hours of exposure (the longest exposure time we are comfortable with without feeding) to allow for selenium accumulation.
3. After 48 hours of exposure take a final water sample (100 μ L) for determination of Se-75.
4. Carefully remove individual artemia with plastic transfer pipette and transfer them (individually) through a series of 3 rinses (10-15 mls each) of fresh media in a 6-well plate prior to placing them in individual gamma counting vials containing 3 mls of Se-75 free GSL media.
5. Pass these samples through a gamma counter for Se-75 determination in the live artemia.
6. After gamma counting, transfer individual artemia to separate 50-ml falcon tubes containing 30 ml GSL media. Feed animals daily and renew GSL media every other day.

7. At regular intervals (days apart), repeat steps 4-6 until significant depuration has been achieved.
8. Once depuration has been achieved and after a final rinse, carefully blot individuals dry on a paper towel and determine mass on weighing paper to nearest 10 μg .
9. Dilute the Se-75 stock as appropriate to measure cold Se on the GFAAS; then take three 10- μL samples of this diluted stock to be read on gamma counter.
10. Determine CPMs of all relevant samples on gamma counter: blank, initial water sample, blank, final water sample, 30 individuals, final water sample #2, 5 individuals, and diluted Se-75 stock.
11. Measure cold Se on GFAAS and determine specific activity of Se-75 stock by dividing: $(\text{cpm/L}) / (\mu\text{g Se/L}) = \text{cpm}/\mu\text{g Se}$.
12. Calculate Se accumulation and depuration according to: $(\text{cpm/individual}) / (\text{cpm}/\mu\text{g}) = \mu\text{g Se/individual}$.